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# DNA barcoding of Mangroves using Ribosomal ITS Marker in Rhizophoraceae

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## ABSTRACT

DNA barcode is a very short, standardized DNA sequence in a well-known gene. It provides a way to identify the species to which a plant, animal or fungus it belongs. One of the most popular sequences identification is achieved by comparing DNA sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal cistron. DNA barcoding is a new concept of identifying unknown organisms based on species DNA regions. The nuclear ribosomal Internal Transcribed Spacer region is widely used as a DNA bacoding marker to characterize the diversity and composition of plant groups. The ITS region has been heavily used in both molecular methods and ecological studies, due to its high degree of interspecific variability, conserved primer sites and multiple copy nature in the genome. Primers have long been available for the nuclear ITS (Internal transcribed spacer) rDNA region which are now commonly used for plant identification.

**Keywords:** Mangrove, *Rhizophora*, ITS, rDNA**.**

## I. INTRODUCTION

The genus, *Rhizophora* L. belongs to the family Rhizophoraceae. Members of the genus are referred to as red Mangrove and are the most abundantand important among the mangrove ecosystem. Red mangroves are found closest to the water and are the most recognizable mangrove trees due to their "walking" prop roots. These prop roots not only provide support and stability for the tree, but they also have pores (called lenticels) which supply oxygen to the buried roots[1].

The concept of DNA barcoding proposes that effective, broad identification systems can be based on sequence based diversity in short, standardized gene regions[2,3,4]. In plants, the majority of sequenced-based molecular phylogenetic studies, particularly in the early years, were based exclusively on genes and spacers from the plastid genome.[5,6,7,8,9]In addition, several chloroplast genes and intergenic regions are employed however they are often not variable enough to yield well resolved and reliable phylogenies. To overcome this problem, the use of non-coding regions of single or low-copy nuclear genes has been promoted.[10]Accordingly, this tool, the internal transcribed spacer (ITS) region of the 18S–5.8S–26S nuclear ribosomal cistron, now is extensively employed around the globe, having first been utilized scarcely a decade ago.[11]

## II. METHODS AND MATERIAL

Plant samples were collected from kumbalam ($9^0$5' N: $76^0$12' E) of Ernakulum district in Kerala state. The plant materials were authenticated from Botanical Survey of India. The sequences obtained using barcode markers: *rbcL* and *matK* were submitted to the NCBI GenBank. (Accession numbers indicated in Table 1)

The DNA extraction carried out using Nucleospin Plant II Kit (Macherey-Nagel). PCR amplification was performed using forward and reverse primers (Table 2&3). Sequencing of PCR product was carried out in Gene Amp PCR system 9700. Obtained DNA sequence was subjected to NCBI.

Table 1: Details of the mangrove species used in the present study with family, status, life form, voucher

number and GenBank accession numbers obtained after sequence submission.

| No | Specimen | Family | Status | form | |
|---|---|---|---|---|---|
| 1 | *R. apiculata* | Rhizophoraceae | TM | Tree | KX231337 |
| 2 | *R.mucronata* | Rhizophoraceae | TM | Tree | KX231338 |

Table - 2 Primers used

| | | | |
|---|---|---|---|
| *ITS* | ITS-5F | Forward | GGAAGTAAAAGTCGTAACAAGG |
| | ITS-4R | Reverse | TCCTCCGCTTATTGATATGC |

Table -3 PCR amplification profile

| step | Time(sec) | | Cycles | |
|---|---|---|---|---|
| | ITS | | ITS | |
| Initial denature | 98 | 30 | 1 | 1 |
| Denature | 98 | 5 | 40 | 40 |
| Annealing | 58 | 10 | 40 | 40 |
| Extention | 72 | 15 | 40 | 40 |
| Final Ext. | 72 | 60 | 1 | 1 |
| Hold | 4 | ∞ | _ | _ |

## III. RESULTS AND DISCUSSION

In the present study partial genome sequencing of ITS were helpful to discriminate *R. apiculate* and *R. mucronata*. In *Rhizophora* species the base pair variation show in the positions 251,278,406,408,414,447,516,533,629,635in ITS region were noticed. (Plate-1,2). The sequencing failure of rbcL gene were noted in the mangrove species of *R. apiculata* due to incomplete degradation of DNA, slow drying under moist conditions and high level of salt content. In such cases, the possible solution is to search for molecular techniques that support the extraction and sequencing of mangrove plant species.[12] Another study revealed rbcL+ matKloci were not able to adequately discriminate *Rhizophora* species carried out works in Goa.[13]

1 TTTCCGTAGG TGAACCTGCG GAAGGATCAT
TGTCGAAAGC TCTGCCCAGC AGAACGACCC
61 GCGAACACGT TTCACCACGC
GGCGCCGGGC GATCGGCGGT
CCACGGGCCGTCGCGCGCCC
121 CGTCTGCCGA GGGGGCGATC
GCACGTCGGT CCTCCCACGG
CGCAAACAACAAACCCCGG
181 CGCAAGTCGC GCCAAGGAAA
TCAAAGATCG AAAGGCAGCG
CCCCGTCGCCGGGAACGG
241 CGCGCGGGCG GGGCGCTGCG
ACTCCACGAT GAGAATCCCA
ACGACTCTCGCAACGGATA
301 TCTCGGCTCT CGCATCGATG
AAGAACGTAG CGAAATGCGA TACTTGGTGT
GAATTGCAGA
361 ATCCCGCGAA CCATCGAGTC
TTTGAACGCA AGTTGCGCCC GAAGCAATCC
CGCCGAGGGC
421 ACGTCTGCCT GGGTGTCACA
CAACATTGCC CACCCCACCT CCGAACCCTC
GGGGGCACGG
481 TCGGTGGCGG AAGATGGCCT
CCCGTGAGCA CGGCCCCGCG
GTTGGCCCAAAACCAAGTC
541 CCCGACGACG TTCGCCTCGA
CAACGGTGGC TGAGCGACCC
TCGCACAGTGTCGCGCGGCG
601 CGTCGTCGCC CCGGACCCGG
AAGAACCCCA CCCTTTCTCG CAACGCGACC
CCAGGTCAGG
661 CGAGACTACC CGCTGAGTTT AA 682

Plate-1 *R.apiculata*. 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

1 TTTCCGTAGG TGAACCTGCG GAAGGATCAT
TGTCGAAAGC TCTGCCCAGC AGAACGACCC

61 GCGAACACGT TTCACCACGC GGCGCCGGGC GATCGGCGGT CCACGGGCCG TCGCGCGCCC
121 CGTCTGCCGA GGGGGCGATC GCACGTCGGT CCTCCCACGG CGCAAACAAC AAACCCCGG
181 CGCAAGTCGC GCCAAGGAAA TCAAAGATCG AAAGGCAGCG CCCCGTCGCC GGGAACGG
241 CGCGCGGGCG AGGCGCTGCG ACTCCACGAT GAGAATCTCA ACGACTCTCG CAACGGATA
301 TCTCGGCTCT CGCATCGATG AAGAACGTAG CGAAATGCGA TACTTGGTGT GAATTGCAGA
361 ATCCCGCGAA CCATCGAGTC TTTGAACGCA AGTTGCGCCC GAAGCGACCC CGCTGAGGGC
421 ACGTCTGCCT GGGTGTCACA CAACATCGCC CACCCCACCT CCGAACCCTC GGGGGCGCGG
481 TCGGTGGCGG AAGATGGCCT CCCGTGAGCA CGGCCTCGCG GTTGGCCCAA AAACCAAGTC
541 CCCGACGACG TTCGCCTCGA CAACGGTGGC TGAGCGACCC TCGCACAGTG TCGCGCGGCG
601 CGTCGTCGCC CCGGACCCGG AAGAACCCAA CCCTATCTCG CAACGCGACC CCAGGTCAGG
661 CGAGACTACC CGCTGAGTTT AA 682

Plate-2 .*R. mucronata* . ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.

In eukaryotes, the genes encoding ribosomal RNAs are organized in arrays which contain repetitive transcriptional units involving 16 – 18S, 5.5S, and 23 – 28S rRNAs, two transcribed intergenic spacers ITS1 and ITS2. These units are transcribed by RNA polymerase I. The product of RNA polymerase I is processed in the nucleolus, where ITS1 and ITS2 are excised and three types of rRNAs produced. In eukaryotic genomes the ITS regions vary greatly in size and sequence. In mice, ITS1 and ITS2 have lengths 999 bp and 1089 bp, respectively. In the parasitic protzoan Giardia lamblia the lengths of these regions are only 41 bp and 55bp [14].The longest ITSs were found in Coleoptera - the lengths vary between 791 and 2572 kb [15]. In fungi, ITS1 and ITS2 were studied not only with regard to phylogenetics and taxonomy, but also in connection to development of diagnostic strategies for species identification in medicine and ecology[16,17] .*Saccharomycescerevisiae*and *Schizosaccharomyce spombe*are widely used as eukaryotic model organisms, thereforethe lengths and structures of their ITSs are well described. In *S.cerevisiae*the ITS1 spans 361 bp and ITS2 is 232 bp long, In *S.pombe*the corresponding lengths are 412-420 bp and 300 bp[18].

## IV. CONCLUSION

Scheming and developing taxonomic specific DNA barcoding methods has been an happening process for researchers around the world. DNA barcoding methods are always upgrade and it is important to remain current with the identification of new primers and methods as new taxonomic groups are studied and methods are developed. In this paper we have concluded that ITS region is very much effective for DNA barcoding of plant groups and it is the major tools for identification of known as well as unknown organisms.

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